Aerobic soil metabolism of metsulfuron-methyl

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Abstract: A laboratory study was conducted to determine the degradation rates and identify major metabolites of the herbicide metsulfuron-methyl in sterile and non-sterile aerobic soils in the dark at 20°C. Both [*phenyl*-U-^{14}C]- and [*triazine*-2-^{14}C]metsulfuron-methyl were used. The soil was treated with [^{14}C]metsulfuron-methyl (0.1 mg kg^{-1}) and incubated in flow-through systems for one year. The degradation rate constants, DT_{50}, and DT_{90} were obtained based on the first-order and biphasic models. The DT_{50} (time required for 50% of applied chemical to degrade) for metsulfuron-methyl, estimated using a biphasic model, was approximately 10 days (9–11 days, 95% confidence limits) in the non-sterile soil and 20 days (12–32 days, 95% confidence limits) in the sterile soil. One-year cumulative carbon dioxide accounted for approximately 48% and 23% of the applied radioactivity in the [*phenyl*-U-^{14}C] and [*triazine*-2-^{14}C]metsulfuron-methyl systems, respectively. Seven metabolites were identified by HPLC or LC/MS with synthetic standards. The degradation pathways included *O*-demethylation, cleavage of the sulfonylurea bridge, and triazine ring opening. The triazine ring-opened products were methyl 2-[[[[[[[(acetylamino)carbohyl]amino]carbonyl]amino]carbonyl]-amino]sulfonyl]benzoate in the sterile soil and methyl 2-[[[[[amino[(amino-carbonyl)imino]methyl] amino]carbonyl]amino]sulfonyl]benzoate in the non-sterile soil, indicating that different pathways were operable.

Keywords: metsulfuron-methyl; degradation; soil; metabolites; DT_{50}; synthesis

1 INTRODUCTION

Metsulfuron-methyl is a low-use-rate sulfonylurea (SU) herbicide used for broadleaf weed control in cereals, pasture, plantation crops and non-crop situations.[1–3] It is one of the SU herbicides which were discovered in the mid-70s by Dr George Levitt at DuPont. It inhibits the enzyme acetolactate synthase (ALS), also known as acetohydroxy acid synthase (AHAS), which stops plant cell division by inhibiting biosynthesis of the essential amino acids valine and isoleucine.[1–3]

Pesticide transport in soils has received increasing attention during recent years because of concern over potential effects on surface and ground water quality. Soil mobility and degradation are the most important processes that determine the fate of pesticides in soils. Extensive research has been carried out on the mobility of SU herbicides both in laboratory and field studies.[4–7]

Sulfonylurea herbicides degrade in soil primarily by chemical hydrolysis and microbial metabolism and there have been several publications which elucidate the significance of microbial degradation.[3,8–11] Chemical hydrolysis of metsulfuron-methyl has been shown to be very rapid at low pH.[11–13] The hydrolysis half-life at 45°C increased from 2.1 days at pH 5 to 33 days at pH 7.[11] The degradation rate of metsulfuron-methyl in soils is affected by soil temperature, moisture, pH, and microbial viability. The half-life of metsulfuron-methyl is shorter at higher temperatures and moisture contents and ranges from 2.5 days (soil conditions: pH 3.1, 35°C, 80% field water holding capacity (FC)) to 36 days (soil conditions: pH 5.7, 10°C, 60% FC) depending on these factors.[10,14] The degradation rate of metsulfuron-methyl has been positively correlated with microbial biomass.[6] Although several researchers have reported the effects of environmental conditions on the degradation rates of metsulfuron-methyl, there have been only a few attempts to identify the degradation products. Some aqueous hydrolysis products, plant metabolites, and degradation products of metsulfuron-methyl in soil minerals and humic acids have been identified.[12,15–17]

The purpose of this laboratory study was to determine the degradation rate of ^{14}C-labeled metsulfuron-methyl in sterile and non-sterile soils, to clarify the role of microbial metabolism in degradation, to identify major metabolites, and to propose metabolic pathways of metsulfuron-methyl in an aerobic soil.

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2 MATERIALS AND METHODS

2.1 Chemicals and reference standards

Two separate ^{14}C-radiolabeled metsulfuron-methyls (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate) (**1**, Fig 1) were synthesized at DuPont New England Nuclear (NEN) Research Products (Boston, MA). One was labeled uniformly in the phenyl ring [*phenyl*-U-^{14}C] with a specific activity of 1.42 MBq mg^{-1} (38.28 μCi mg^{-1}), and the other was labeled at the 2-carbon position in the triazine ring [*triazine*-2-^{14}C] with a specific activity of 1.85 MBq mg^{-1} (49.87 μCi mg^{-1}). Both ^{14}C-labeled compounds had radiochemical purities greater than 99% as determined by high performance liquid chromatography (HPLC). Two solutions at concentrations of 10 mg litre^{-1} (one for each ^{14}C-label) were prepared by dissolving the ^{14}C-labeled metsulfuron-methyl in water.

All organic solvents were HPLC grade. All other chemicals were reagent grade or better. Water was purified using a Milli-Q® water purification system.

Unlabeled reference standards (**2**–**9**, Fig 1) were synthesized by DuPont Agricultural Products, E I du Pont de Nemours and Company. The detailed syntheses of unique metabolites **4**, **8** and **9** are described below. Other reference standards were prepared by generally known methods or were commercially available.

2.1.1 *Synthesis of methyl 2-[[[[[amino[(aminocarbonyl)imino]methyl]amino]-carbonyl]amino]sulfonyl]benzoate* (*4*)

Free base guanylurea was freshly prepared from commercially available guanylurea sulfate by heating a slurry of the material in ethanol with sodium ethoxide (0.75 eq) for 2 h at 60°C. After cooling, the mixture was filtered and the filtrate evaporated. Traces of ethanol were removed from the solid residue by addition and evaporation of benzene twice, then chloroform three times, to leave a solid residue of guanylurea, mp 104–107°C. To a suspension of guanylurea (1.0 g, 9.8 mmol) in anhydrous acetonitrile (75 ml) was added, dropwise, a solution of 2-(methyoxycarbonyl)benzenesulfonyl isocyanate (2.5 g; 10.3 mmol) in acetonitrile (15 ml) over a half-

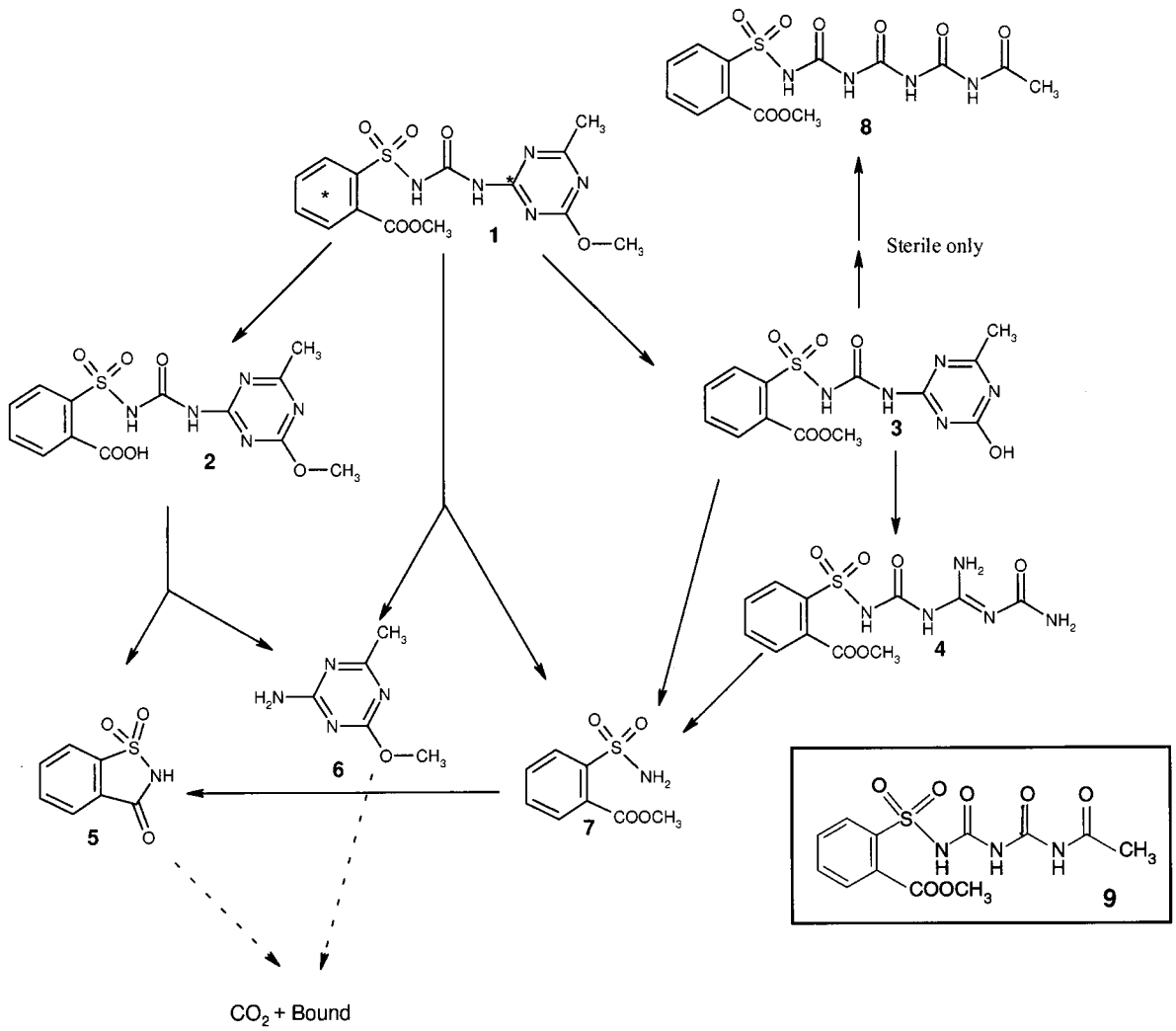

Figure 1. Proposed metabolic pathways of metsulfuron-methyl in soil (* denotes site of ^{14}C). The inset shows **9**, a possible alternative to **4** (see Section 3.4.2).

hour period at room temperature (*c.* 20°C) under nitrogen. After 24 h the suspension was filtered and the solids washed successively with acetonitrile, ether, then dichloromethane (×3). Trituration of these solids with warm acetonitrile (50°C) and filtration with dichloromethane rinse afforded 1.42 g (42%) of **4** as a white powder, mp > 260°C: [^{1}H]NMR [dimethylsulfoxide(DMSO)-d_6, 360 MHz] δ 3.76 (s, 3H), 7.42 (m, 2 × NH), 7.56 (m, 3H), 7.97 (d, 1H), 8.75 (br, NH), 10.40 (br, NH); LC/MS (electrospray, negative mode) *m/z* 342 (40%, $[M-H]^-$, 299 (100%), 214 (20%), 182 (50%), (positive mode) *m/z* 344 (100%, MH^+).

2.1.2 Synthesis of methyl 2-[[[[[[[(acetylamino)-carbonyl] amino] carbonyl]amino]carbonyl]amino]-sulfonyl]benzoate (***8***)

To a sample of acetylbiuret (0.24 g; 1.65 mmol)[18] was added a solution of 2-(methoxycarbonyl)benzenesulfonyl isocyanate in xylenes (340 g litre^{-1}; 2.5 ml). The mixture was heated on a steam bath under a stream of nitrogen to evaporate the solvent. After 5 h, the mixture was cooled and triturated with ethyl acetate and the solids filtered. This material was further purified by trituration with warm (40°C) acetonitrile, filtered and dried in vacuum to afford 0.30 g (47%) of **8** as a white crystalline powder, mp 224–226°C: [^{1}H]NMR (DMSO-d_6, 300 MHz) δ 2.09 (s, 3H), 3.85 (s. 3H), 7.80 (m, 3H), 8.11 (d, 1H), 10.26 (s br, NH), 10.97 (m br, 3 × NH); LC/MS (electrospray, negative mode) *m/z* 385 (100%, $[M-H]^-$).

2.1.3 Synthesis of methyl 2-[[[[[(acetylamino)-carbonyl]amino] carbonyl]amino]sulfonyl]benzoate (***9***)

This substance (see Fig 1) was prepared from acetylurea by an analogous procedure to that described above for **8**. Data for **9**: mp 184–189°C; [^{1}H]NMR (DMSO-d_6, 300 MHz) δ 2.08 (s, 3H), 3.85 (s, 3H), 7.80 (m, 3H), 8.12 (d, 1H), 10.58 (s, NH), 11.06 (s, NH); LC/MS (electrospray, negative mode) *m/z* 342 (100%, $[M-H]^-$).

2.2 Soil

Matapeake silt loam (pH 5.2; 1.8% organic matter; 16.8% clay, 55.6% silt, 27.6% sand; cation exchange capacity 7.0 meq 100 g^{-1} soil) was collected from the top 15 cm of a field located in Middletown, Delaware. The fresh soil was passed through a 2-mm sieve and thoroughly homogenized before the application of metsulfuron-methyl.

2.3 Flow-through systems

2.3.1 Non-sterile system

The soil (equivalent to 50 g, oven-dry weight) was weighed into Pyrex® glass flasks (250 ml). The respective ^{14}C-labeled metsulfuron-methyl solutions (10 mg litre^{-1}) were applied to the soil in each of the designated flasks to produce a soil concentration of 0.1 mg kg^{-1}. Two flasks treated with each of the two ^{14}C-labeled metsulfuron-methyl solutions were considered as replicates. Metsulfuron-methyl and the soil in each flask were mixed by hand shaking. Sufficient Milli-Q® water was added to each flask to adjust the moisture content of the soil to approximately 75% of FC (FC = 17.4%). The soil moisture content in each flask was maintained at 75% of FC by addition of water at least monthly throughout the study. Additionally, four flasks were treated with [^{14}C]metsulfuron-methyl (two flasks per ^{14}C-label) at a concentration of 1 mg kg^{-1} (high dose) in order to generate sufficient quantity for metabolite identification. The high-dose flasks were treated and incubated in the same manner as the flasks containing 0.1 mg kg^{-1} metsulfuron-methyl. Flasks with treated soil were sampled for soil extraction at days 0, 1, 3, 7, 14, and 21, and at months 1, 2, 3, 4, 6, 9, and 12.

2.3.2 Sterile system

The flasks were prepared as described above for the non-sterile system except that each flask contained the sterile soil (50 g dry weight equivalent) autoclaved (at 15 psi and 120°C) for 1 h on four consecutive days. The moisture content of the sterile soil was adjusted to 75% of FC with sterilized water (0.2 μm filter). The sterile system was connected as described in Section 2.3.4 with the addition of a 0.2 μm filter disk (Gelman ACRO 50 PTFE) inserted before the flask to maintain sterility. For each [^{14}C]metsulfuron-methyl treatment, two flasks were sampled, one for extraction to analyze for parent and metabolites, and the other for plate counts to determine sterility, at day 0 and at months 1, 2, 4, 5, 6, 9, and 12.

2.3.3 Control and biomass system

The flasks were dosed with non-radiolabeled metsulfuron-methyl solution at the same concentration (0.1 mg kg^{-1}) and maintained under identical conditions to the non-sterile soil flasks. The soils in the flasks were only sampled at the termination of the study to determine the biomass and microbial plate counts. Soil biomass and microbial plate counts were also conducted at the initiation of the study prior to application of metsulfuron-methyl. Biomass was determined by Anderson-Domsch's glucose-substrate induced respiration method.[19] Bacteria and fungi were enumerated using plate-count agar and Sabaroud agar (Difco laboratories), respectively.

2.3.4 Flow-through systems

In both the non-sterile and the sterile systems (Sections 2.3.1 and 2.3.2), each flask was fitted with a glass impinger with an air inlet and outlet tubing connected to a series of six traps. The soil flask inlet was connected to an outlet of a water bottle to provide humidified air (an inlet of the water bottle was open to the air). The traps used in order of proximity to the flask were a blank overflow trap, two

ethylene glycol traps (20 ml), a polyurethane foam plug trap to collect any organic volatiles, and two potassium hydroxide traps (1 N; 20 ml) containing phenolphthalein indicator (to indicate carbon dioxide saturation of traps) to collect [^{14}C]carbon dioxide evolved from the [^{14}C]metsulfuron-methyl. The outlet of the last potassium hydroxide trap was connected to a vacuum manifold which was used to regulate the air flow in the system.

In the control and biomass system (Section 2.3.3), all the flasks were connected in series and the last flask was connected to three traps (ethylene glycol, foam plug, and potassium hydroxide) to permit measurement of background radioactivity. The outlet of the potassium hydroxide trap was connected to a vacuum manifold which was used to regulate the air flow in the system. The water bottle was placed before the soil flasks to provide humidified air as in the sterile and non-sterile systems.

In order to monitor the formation of [^{14}C]carbon dioxide and ^{14}C-organic volatiles resulting from the degradation of [^{14}C]metsulfuron-methyl, each trap (foam plug, potassium hydroxide, and ethylene glycol traps) was sampled and replaced with fresh solutions or new foam plug monthly and at each soil sampling date. Although no soil sample was taken from the control and biomass system, the trapping solutions were collected in the same manner as for the non-sterile system for background radioactivity.

The connected flow-through systems were maintained in a temperature-controlled chamber at 20°C (±2°C) in the dark under aerobic conditions for one year.

2.4 Analytical methods

2.4.1 Analysis of radiolabeled volatiles

Triplicate aliquots (3 × 1 ml) of the potassium hydroxide and ethylene glycol trap solutions used to trap [^{14}C]carbon dioxide and organic volatiles were combined with scintillation fluid (5 ml) and analyzed for total radioactivity by LSC (liquid scintillation counting, Beckman Instrument Inc). The polyurethane foam plugs were extracted with acetonitrile + 2 M ammonium carbonate (3 × 10 ml, 90 + 10 by volume). Triplicate aliquots (3 × 1 ml) of the extract were analyzed for total radioactivity by LSC.

2.4.2 Soil extraction

At each soil sampling time as described above, two flasks (one from each of the two [^{14}C]metsulfuron-methyl treatments) were taken. A two-step extraction method was used.

Step 1: The soil in each flask was combined with acetonitrile + 2 M ammonium carbonate (100 ml, 9 + 1 by volume) and shaken for 1 h on a platform shaker at room temperature (*c.* 20°C). The solution was centrifuged (approximately 2500 rev min^{-1}) for 15 min and the supernatant was decanted. This extraction was conducted three times and the extracts were pooled. Triplicate aliquots (3 × 1 ml) were analyzed by LSC. If the estimated non-extractable residues (bound residues) were greater than 10% of the applied radioactivity (AR) (bound residues %AR = total applied (100% AR)-%AR in extracts-%AR in traps), extraction step 2 was conducted.

Step 2: After step 1 extraction, the soil sample was combined with methylene chloride + methanol + 2 M ammonium carbonate (100 ml, 3 + 4 + 1 by volume) and shaken for 1 h at room temperature. The extraction was conducted three times and the extracts were pooled. Triplicate aliquots (3 × 1 ml) were analyzed by LSC. Step 1 and step 2 extracts were pooled and concentrated by vacuum using rotary evaporation. The residues were dissolved in water. Triplicate aliquots were analyzed by LSC to determine the total radioactivity in the extract. The extract was also analyzed by HPLC to determine concentrations of metsulfuron-methyl and its metabolites.

2.4.3 Analysis of soil extracts

Soil extracts were analyzed using a HPLC equipped with both a UV-Vis detector and an on-line radiochemical detector (Ramona, Raytest Inc). HPLC method 1 used a Zorbax Rx-C8 column (250 × 4.6 mm, 5 μm) and a gradient with mobile phase of A, water (pH = 2.3 with 1 g litre^{-1} phosphoric acid), and B, acetonitrile (from 0 to 5 min, 0% B; at 15 min, 15% B; at 25 min, 40% B; at 30 min, 100% B; from 30 to 35 min, 100% B, at 38 min, 0% B). HPLC method 2 used a PRP-1 column (305 × 7.0 mm, 10 μm) with the same mobile phase as HPLC method 1 but a different gradient (from 0 to 3 min, 10% B; at 10 min, 20% B; at 20 min, 40% B; at 30 min, 90% B; at 35 min, 10% B). The mobile phase flow rate was 1.5 ml min^{-1} in both methods. HPLC method 1 was used for all the sample analyses and HPLC method 2 was used for confirmatory analyses. Selected soil extracts were spiked with unlabeled reference standards of **2** (2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]carbonyl]amino]sulfonyl]benzoic acid, common name: metsulfuron), **3** (methyl 2-[[[[(4-hydroxy-6-methyl-1,3,5-triazine-2-yl)amino]-carbonyl]amino]sulfonyl]benzoate, trivial name: *O*-desmethyl metsulfuron-methyl), and **7** (methyl 2-(aminosulfonyl)benzoate, trivial name: benzene sulfonamide) and analyzed using both HPLC methods described above to confirm the identities of metabolites. Metabolite **6** (4-methoxy-6-methyl-1,3,5-triazine-2-amine, trivial name: triazine amine) was isolated from the two-month samples (high dose) and analyzed using both HPLC methods to confirm its presence by retention time matches between the sample and ^{14}C-labeled reference standard. Metabolites **4**,**5**, and **8** were analyzed by LC/MS for identification under the conditions described below.

An LC (equipped with Micro-Tech Scientific ultra-plus gradient pump, a Zorbax SB-C18 column

(150 × 1 mm, 3.5 μm), acetonitrile with 0.5 ml litre^{-1} formic acid + water with 0.5 ml litre^{-1} formic acid as mobile phase (3 + 97 by volume as initial condition)) was connected to both a Ramona radiochemical detector and a mass spectrometer (MAT 900, Finnigan) interfaced with a MAT 900 series atmospheric pressure ionization (API) system, electrospray mode (Finnigan). The analyses were made in the negative ion and/or positive ion modes. The MS parameters were as follows: scan range, 45–1000 amu (atomic mass units); ion source temperature, 40°C; electron multiplier voltage, 1.80 kV positive ion, 1.90 kV negative ion, 2.0 kV for accurate mass experiment. CID (collision induced dissociation) capillary and tube lens both offset at 30 volts.

2.4.4 Determination of soil bound residues

All the post-extracted soil samples were air-dried in a laboratory hood. When dry, the samples were homogenized and weighed. Triplicate aliquots were combusted using a Harvey biological oxidizer (Harvey Instrument Inc, model OX 500) and the [^{14}C]carbon dioxide released from the combustion process was trapped in ^{14}C-cocktail scintillation fluid (15 ml) and radioactivity was measured by LSC.

2.4.5 Fractionation of soil bound residues

Soil bound residues were fractionated with strong base and acid into three fractions: humin, humic acid (HA), and fulvic acid (FA).[20] Radioactivity in the three fractions was determined by LSC. This fractionation procedure was conducted only on four-month samples. The post-extracted soil was mixed with aqueous sodium hydroxide (1 M; 100 ml) and shaken overnight on a platform shaker at room temperature. The solid phase was separated from the aqueous phase by centrifugation, and washed with aqueous sodium hydroxide (1 M; 2 × 20 ml). The combined aqueous sodium hydroxide extract was adjusted to 150 ml and three aliquots (3 × 2 ml) were analyzed by LSC. The solid (humin fraction) was combusted in a Harvey biological oxidizer and the [^{14}C]carbon dioxide released from the combustion process was trapped in ^{14}C-cocktail scintillation fluid (15 ml) and radioactivity in the humin fraction was measured by LSC. The sodium hydroxide extract was acidified to pH 2 with concentrated hydrochloric acid to precipitate the humic acid fraction (HA). The radioactivity of the remaining supernatant (FA) was determined by LSC. The HA precipitate was redissolved in aqueous sodium hydroxide (1 M; 25 ml) and the solution radioactivity (HA fraction) was determined by LSC.

3 RESULTS AND DISCUSSION

3.1 Soil viability and sterility

3.1.1 Soil viability in the control and biomass system

Measurements of biomass and microbial plate counts conducted at initiation and termination of the study indicated that the soil micro-organisms remained viable throughout the study in non-sterile flasks (Table 1). The dynamics of microbial biomass and population after application of metsulfuron-methyl were not monitored in this study. An earlier study[21] demonstrated that application of metsulfuron-methyl would not adversely affect soil microbial biomass and population once soil microbes adapted to the presence of metsulfuron-methyl.

3.1.2 Soil sterility in the sterile system

Results from plate counts indicated that soil remained sterile for six months. After six months, the system became contaminated, since microbial colonies were seen in the bacteria and fungi plate media for both ^{14}C-labeled metsulfuron-methyl treatments (data not shown). Therefore, only data up to six months were used to interpret results in the sterile system.

3.2 Recovery and distribution of ^{14}C-radioactivity

Data on the recovery and distribution of radioactivity from the non-sterile soil treated with the [^{14}C-*phenyl*]- or [^{14}C-*triazine*]-labeled metsulfuron-methyl are presented in Fig 2. The total ^{14}C recovery was 96.7% AR (±4.4) for the [^{14}C-*phenyl*] label and 98.2% AR (±2.9) for the [^{14}C-*triazine*] label. In the sterile soil, the ^{14}C recovery ranged from 91% AR to 110% AR indicating that mass balance was maintained.

3.2.1 Mineralization

In the non-sterile system, the rate of [^{14}C]carbon dioxide evolution was faster in the soil treated with [*phenyl*-U-^{14}C]metsulfuron-methyl (48.2% AR as carbon dioxide at 12 months) than the soil treated with [*triazine*-2-^{14}C]metsulfuron-methyl (22.8% AR as carbon dioxide at 12 months) (Fig 2). The different rates of mineralization indicate that the phenyl moiety is more susceptible to microbial attack than the triazine moiety of the molecule. In the sterile system, there was no detectable carbon dioxide (detection limit of 0.3% AR) evolved for six months (data not shown).

Table 1. Soil viability test

Parameters	*Microbes (cfu g^{-1}) × 10^5*	
	Study initiation	*Study termination (1 yr)*
Bacteria	8.19 (±6.21)	172 (±23)
Fungi	0.25 (±0.06)	6.83 (±0.85)
Biomass (mg C g^{-1})	0.104	0.116

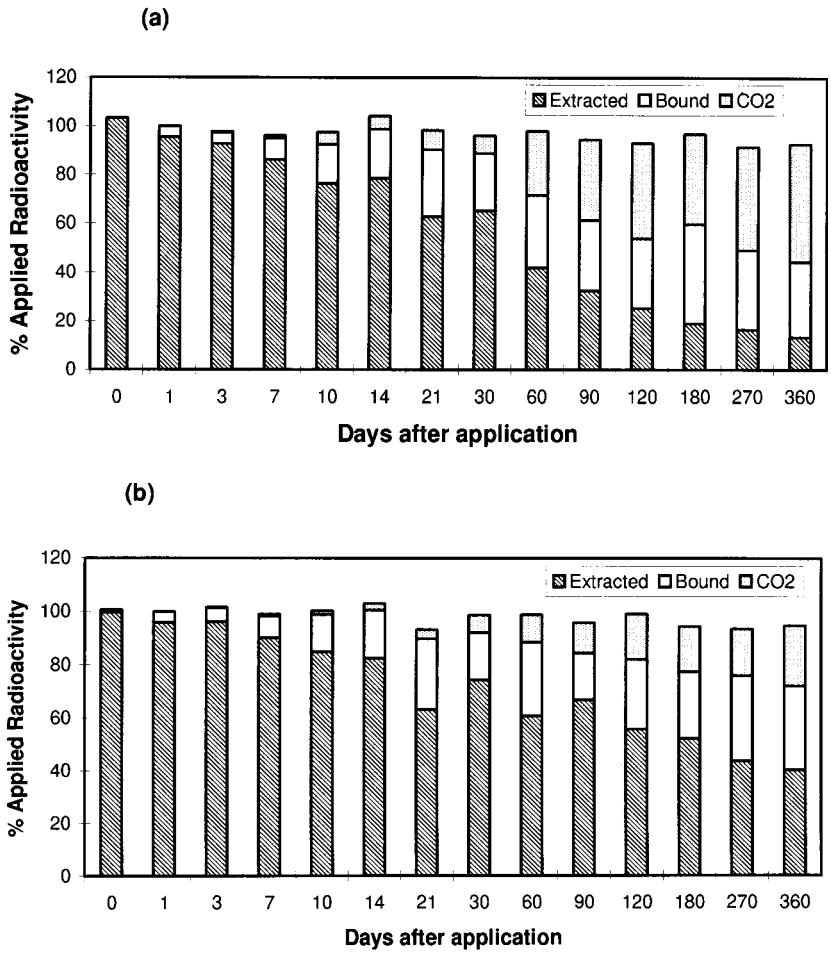

Figure 2. Mass balance of (a) [*phenyl*-U-^{14}C] and (b) [*triazine*-2-^{14}C] labeled metsulfuron-methyl in the non-sterile soil.

3.2.2 Soil bound residues

In this study, we found approximately two-fold more ^{14}C-soil bound residues in the non-sterile than in the sterile soils. Therefore, we believe that microbial activity contributed to soil bound residues. Of the radioactivity remaining in the soil (four-month samples), 14.3%, 0.92%, and 9.14% of AR (the phenyl label) were found in humic acid, fulvic acid, and humin fractions, respectively. For the triazine label, 12.5%, 0.43%, and 6.81% of AR were found in humic acid, fulvic acid, and humin fractions, respectively. The results indicated that bound radioactivity was associated mainly with humin and humic acid fractions.

3.3 Rate of degradation of metsulfuron methyl in aerobic soil

Since the sterile soil was contaminated after six months, only data up to six months were used in the calculation of degradation rate constant (k), DT_{50}, and DT_{90} of metsulfuron-methyl for both the sterile and non-sterile soils in order to make a direct comparison between the two.

Both the first-order and biphasic models were used to fit the data (Table 2 and Fig 3) even though the first-order model has been widely used in the estimation of DT_{50} and DT_{90} values. DT_{50} was estimated by using the equation: $DT_{50} = \ln(2)/k$. The degradation rate constant (k) was determined using linear regression of $\ln(c/c_0)$ (c: concentration) over time and was the slope of the linear regression line. Based on the first-order model, the estimated values of DT_{50} (the time required for 50% of applied chemical to degrade) were 30 days (26–35 days, 95% confidence limits) and 43 days (36–51 days, 95% confidence limits) in the non-sterile and sterile soils, respectively (Table 2). These results are similar to those of James *et al*[14] who found half-lives of eight to 36 days (based on the first-order model) in an acidic soil (pH 5.7 and 7.3% OC) at various soil moistures and temperatures.

The result of the interaction effect (treatment × time) using the JMP® Program (JMP®, V3.2.2, SAS Institute Inc, Cary, NC) indicates that the rate constants (k, linear regression slope) were significantly different between the two treatments, sterile and non-sterile soils. Therefore, the DT_{50} values ($=\ln 2/k$) of 30 and 43 days were significantly different between the non-sterile and sterile soils based on the first-order model.

The lack-of-fit test (using JMP® program) indicates that the first-order model is not a good fit. Based on the biphasic model, the estimated DT_{50} was more close to the measured data (Fig 3). The

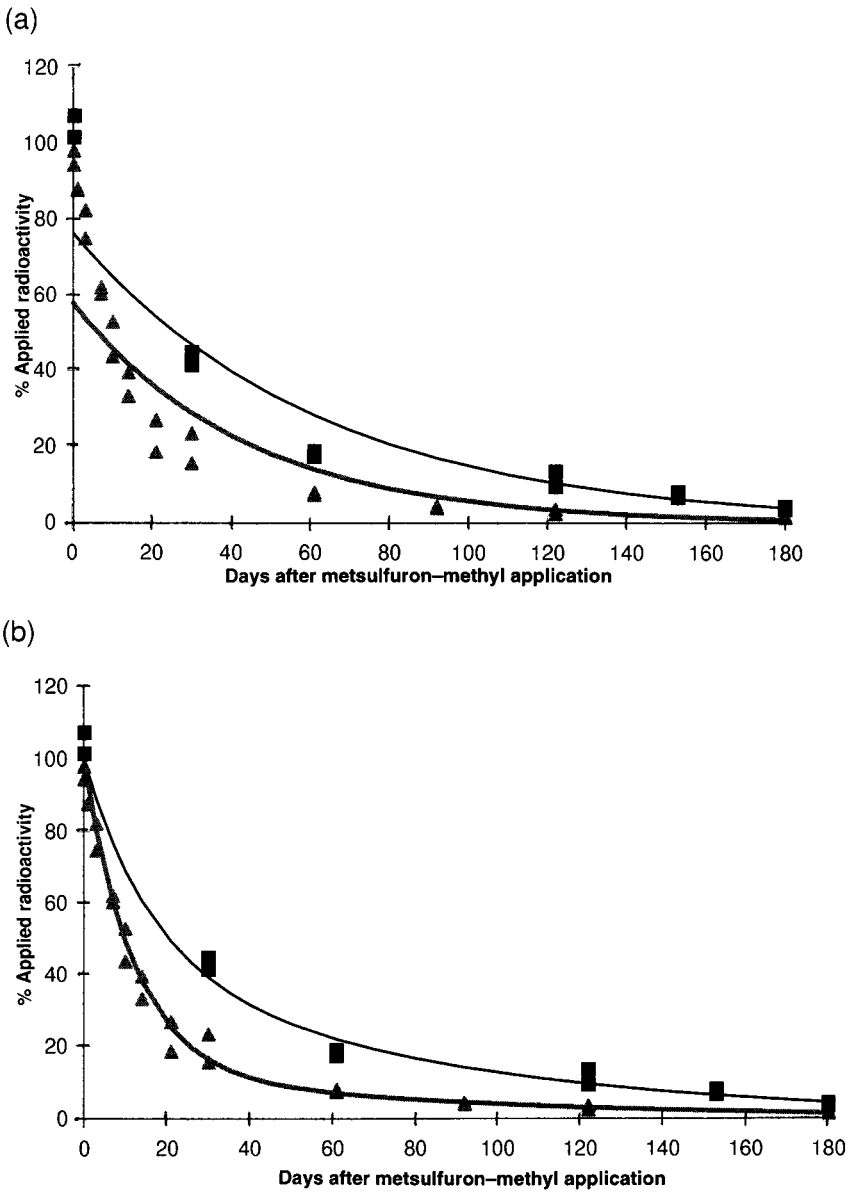

Figure 3. Dissipation of 0.1 mg kg^{-1} of [^{14}C]metsulfuron-methyl over time in soil (pH 5.2), 1.8% OM, 75% FC, and 20°C in the dark, (a) fitted lines based on the first-order model, (b) fitted line based on the biphasic model, (▲) non-sterile soil (■) sterile soil.

DT_{50} values of metsulfuron-methyl were 10 days (9–11 days, 95% confidence limits) and 20 days (12–32 days, 95% confidence limits) in the non-sterile and sterile soils, respectively. The 95% confidence limits of DT_{50} did not overlap between the sterile and non-sterile soils, which suggests that biological degradation (by soil micro-organisms) significantly contributes to the dissipation of this compound. The dissipation of metsulfuron-methyl in soil involves both chemical and microbial processes, and it has been found in other SUs as well.[11,22,23] Degradation of metsulfuron-methyl in an acidic soil environment is relatively rapid because of SU bridge hydrolysis which diminishes in alkaline soils.

3.4 Soil metabolites of metsulfuron-methyl

Six major metabolites (**2**–**7**) were detected by HPLC in the non-sterile soil (Fig 4) and five major metabolites (**3**, **5**–**8**) in the sterile soil (Fig 5). The formation and dissipation of metabolites in the non-sterile soil are presented in Fig 6.

*3.4.1 Identification of **2**, **3**, **6**, and 7 by co-chromatography*

Metabolites **2**, **3**, and **7** were confirmed by comparison of the retention times of unlabeled reference standards with the corresponding radiochemical peaks. Metabolite **6** was confirmed using the radiolabeled standard. Metabolites **3**, **6**, and **7** were found in both sterile and non-sterile soils, while metabolite **2** was found only in the non-sterile soil.

*3.4.2 Identification of metabolite **4** by LC/MS*

Carbamoyl guanidine (**4**) was found only in the non-sterile soil treated with either [*phenyl*-U-^{14}C]- or [*triazine*-2-^{14}C]metsulfuron-methyl. It was isolated and identified using HPLC method 1 (C8 column) and confirmed by LC/MS.

LC/MS–ESI negative ion analysis shows a spectrum with a $[M - H]^-$ m/z 342 corresponding to the

Table 2. Degradation model parameters for metsulfuron-methyl in soil

System	*Model parameters First-order parameters*[a]			DT_{50} (*days*)	DT_{90} (*days*)
	Intercept	*k(slope)*	r^2		
Non-sterile system	4.057	0.0233	0.90	30	99
95% confidence limits[b]				26–35	86–116
Sterile system	4.333	0.0163	0.95	43	141
95% confidence limits				36–51	121–170
	Biphasic model parameters			DT_{50} (*days*)	DT_{90} (*days*)
	a	*k1*	*k2* r^2		
Non-sterile system	13.2	0.0118	0.0837 0.990	10	44
95% confidence limits[c]				9–11	40–50
Sterile system	44.6	0.0126	0.0622 0.977	20	119
95% confidence limits				12–32	99–134

[a] Calculated using data through six months for the non-sterile and sterile soils. $DT_{50} = \ln 2/k$, $DT_{90} = \ln(10)/k$ where k was first-order rate constant and was the slope determined based on linear regression of $\ln(c/c_0)$ versus time. The first-order model: $C = C_0 \cdot e^{-k \times t}$ where, C_0 : initial concentration (mg kg^{-1}); *C:* concentration at time t (mg kg^{-1}); *k:* degradation rate constant.

[b] 95% confidence levels based on linear regression using $\ln(c/c_0)$ versus time.

[c] Biphasic model:

$$C/C_0 = a \cdot e^{-k_1 \times t} + (1 - a) \cdot e^{-k_2 \times t}$$

Where, C_0 : initial concentration (mg kg^{-1}); *C:* concentration at time t (mg kg^{-1}); k_1 and k_2 : slow and fast degradation rate constants. *a:* constant. Constants and confidence limits were determined using JMP® program (SAS Institute, Inc. Cary, NC).

Note: r^2 was reported based on ln scale fitting of both models.

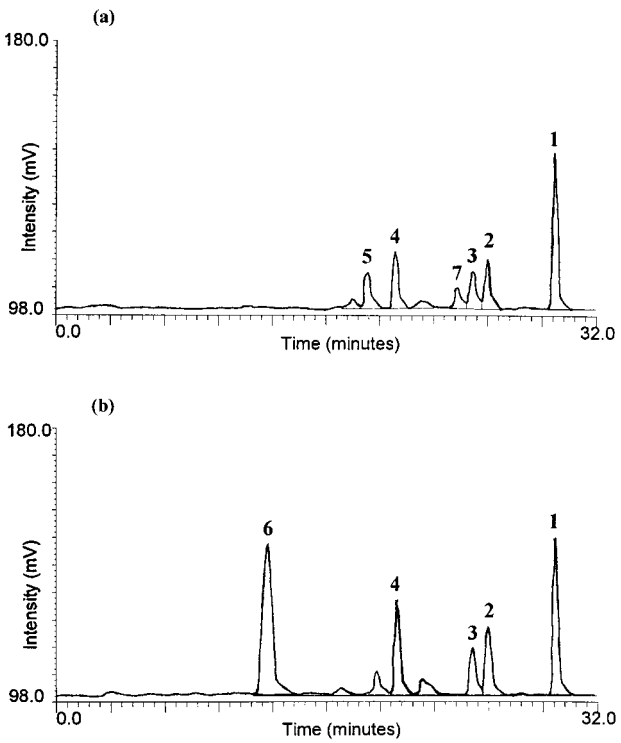

Figure 4. ^{14}C-radiochromatogram of one-month samples in the non-sterile soil ((a) [*phenyl*-U-^{14}C] and (b) [*triazine*-2-^{14}C] labeled metsulfuron-methyl, HPLC conditions: Rx-C8 column, 250 × 4.6 mm, flow rate of 1.5 ml min^{-1}, mobile phase: acetonitrile and acidified water (0.1% H_3PO_4), see Section 2.4.3 for the mobile phase gradient).

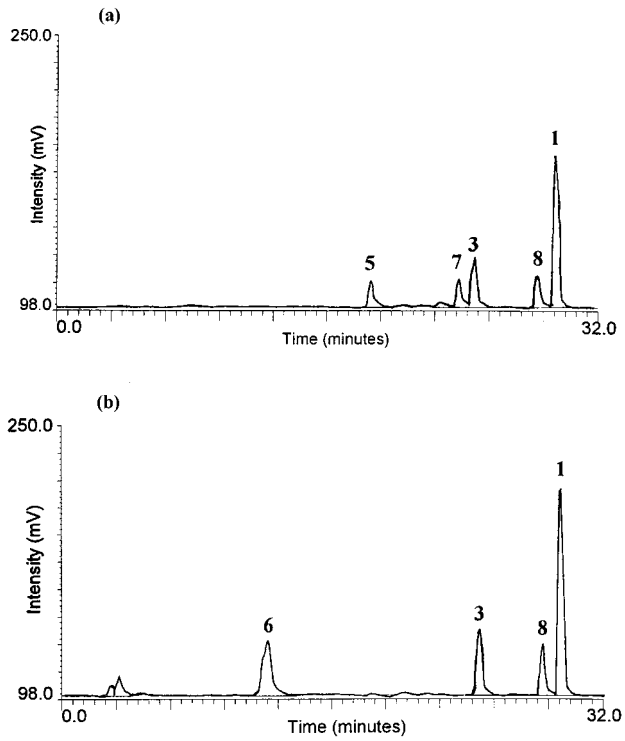

Figure 5. ^{14}C-radiochromatogram of one-month samples in the sterile soil ((a) [*phenyl*-U-^{14}C] and (b) [*triazine*-2-^{14}C] labeled metsulfuron-methyl, HPLC conditions: Rx-C8 column, 250 × 4.6 mm, flow rate of 1.5 ml min^{-1}, mobile phase: acetonitrile and acidified water (0.1% H_3PO_4), see Section 2.4.3 for the mobile phase gradient).

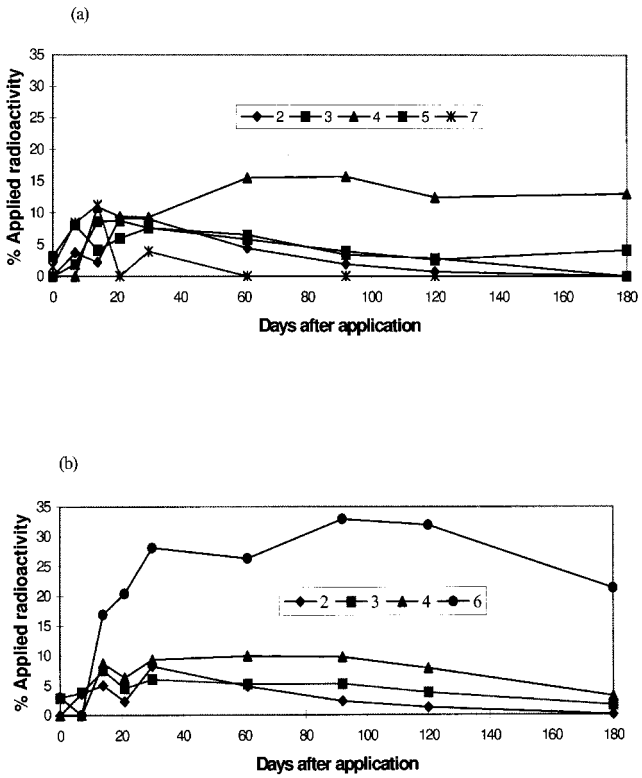

Figure 6. Metabolite profiles of (a) [*phenyl*-^{14}C] and (b) [*triazine*-2-^{14}C] labeled metsulfuron-methyl in the non-sterile soil (pH 5.2, 20°C in the dark; for structure of metabolites, see Fig 1).

on-line ^{14}C peak. The CID mass spectrum (Fig 7) shows fragmentation consistent with the proposed structure (**4**, Fig 1). The ^{14}C ion appears in the spectrum of triazine-labeled metabolite **4** (Fig 7), since this is labeled at one specific carbon atom. The ratio is close to that calculated from specific activity plus small contributions from ^{34}S and ^{18}O.

LC/MS–ESI positive ion analysis shows a spectrum with a MH^+ m/z 344 (100%) and fragmentation m/z 129 (10%) consistent with the proposed structure. LC/MS–ESI(+) high resolution/accurate mass/elemental composition gives an exact mass of 344.0650 with a molecular formula of $C_{11}H_{14}N_5O_6S$. Mass spectral features of the synthetic reference standard of **4** are in good agreement with the samples which contained metabolite **4**. An alternative structure (**9**, see Section 2.1.3) with the same mass but a different elemental composition was synthesized initially but did not match metabolite **4** by CID mass spectrum and accurate mass/elemental composition.

*3.4.3 Identification of metabolite **5** by LC/MS*

Metabolite **5** (1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide, common name: saccharin) was isolated from soil extracts based on HPLC method 1 and confirmed by LC/MS. LC/MS–ESI negative ion analysis shows a spectrum with $[M-H]^-$ m/z 182 corresponding to the on-line ^{14}C peak. A CID spectrum produced no fragmentation of the compound. Analysis of the reference standard of **5** also shows a $[M-H]^-$ m/z 182 and no fragmentation by CID. Therefore, this spectrum is consistent with the structure for **5** and confirmed as saccharin.

*3.4.4 Identification of metabolite **8** by LC/MS and HPLC*

Acetyl triuret (**8**) was found only in the sterile soil treated with either [*phenyl*-U-^{14}C]- or [*triazine*-2-^{14}C]metsulfuron-methyl. It reached approximately 18% AR in the six-month samples (average of the two labels). The radiochemical peak was isolated using HPLC method 1 by fractionation of the column effluent. The retention time of the reference standard matched with the isolated metabolite using the HPLC method 1. The isolated metabolite was further confirmed by LC/MS.

LC/MS–ESI negative ion analysis shows a spectrum with a $[M-H]^-$ m/z 385 with a significant m/z 387 ion in the triazine label corresponding to the on-line ^{14}C peak. This spectrum is consistent with the structure for **8** and the CID spectrum shows a match for the CID mass spectrum of the reference standard (Fig 8) except for the lack of ^{14}C ion (m/z 387) in the reference standard. An aliquot of the isolated sample spiked with the reference standard has coelution of the $[M-H]^-$ m/z 385 mass peak (data not shown).

3.5 Metabolic pathway of radiolabeled metsulfuron-methyl in the soil

Degradation pathways of metsulfuron-methyl in soil under aerobic conditions are proposed in Fig 1. The main metabolites that contained both the triazine and phenyl moieties were **2**, **3**, and **4** in the non-sterile soil, **3** and **8** in the sterile soil. Metabolite **6** contained only the triazine moiety, and metabolites **5** and **7** contained only the phenyl moiety (Fig 1). All the metabolites increased early in the study then dissipated to less than 10% AR by the end of the study (Fig 6), except metabolite **6**, which did not decline rapidly. Both the [*phenyl*-U-^{14}C]- and [*triazine*-2-^{14}C]-metsulfuron-methyl were mineralized to [^{14}C]carbon dioxide extensively in the non-sterile soil.

Cleavage of the sulfonylurea linkage, which has been commonly found in other SU herbicides,[2,8,24,25] results in the formation of metabolites **6** and **7**. Urea bridge cleavage occurs rapidly in acidic aqueous solution in the absence of micro-organisms, but is slow at neutral pH. In the non-sterile soil, this cleavage proceeds through both abiotic bridge hydrolysis as well as microbially mediated hydrolysis.

O-Demethylation of the triazine moiety of metsulfuron-methyl may be a chemical or microbial process (or both) since metabolite **3** was found in both sterile and non-sterile soils. *O*-Demethylation of the ester on the phenyl moiety of metsulfuron-methyl is probably a microbial degradation process

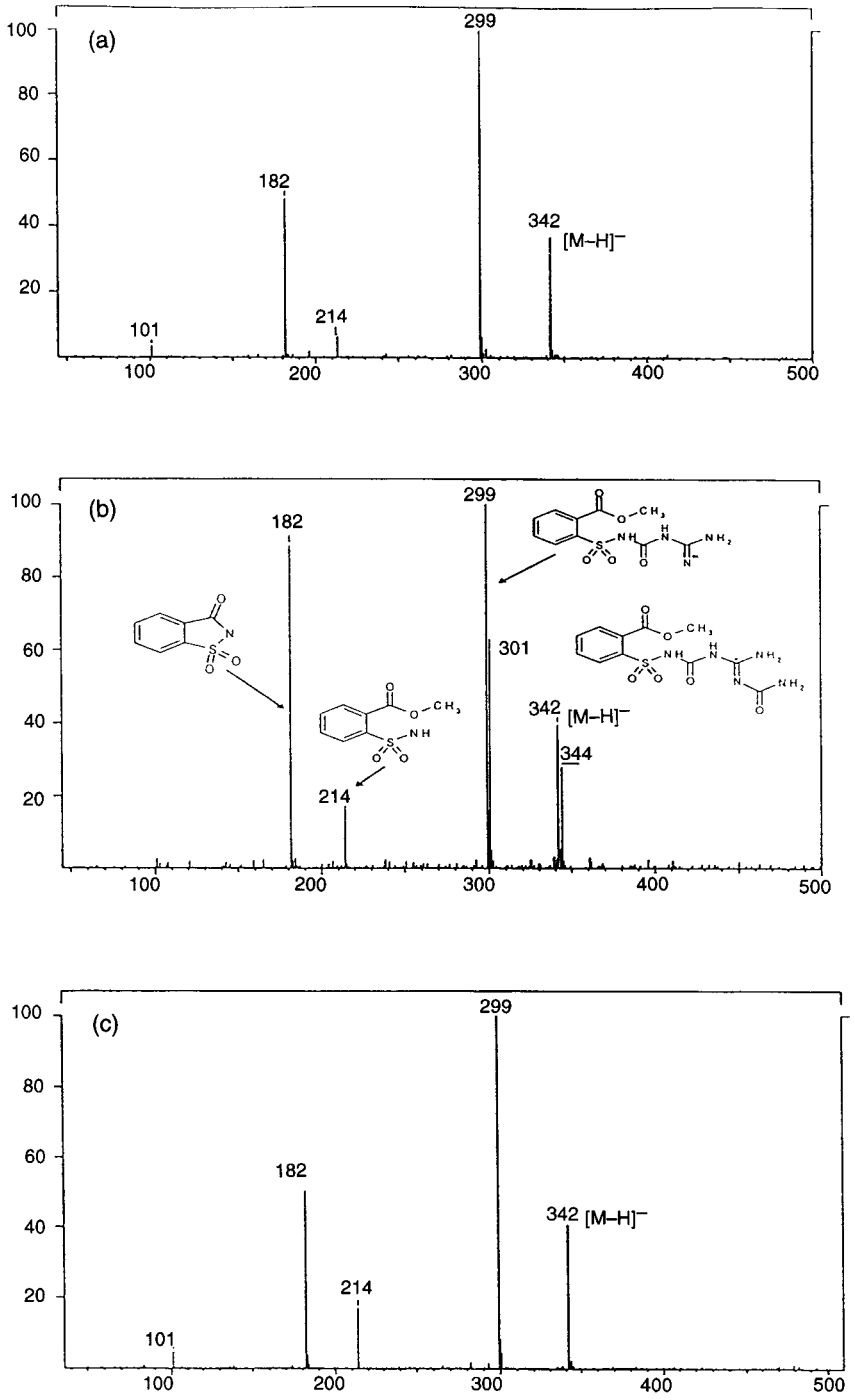

Figure 7. Mass spectrum of metabolite **4** and the reference standard (* denotes site of ^{14}C). (a) Negative ion CID (−30 volts) mass spectrum of metabolite 4 (phenyl label). (b) Negative ion CID (−30 volts) mass spectrum of metabolite **4** (triazine label). (c) Negative ion CID (−30 volts) mass spectrum of the reference standard.

since metabolite **2** was found only in the non-sterile soil.

Metabolite **8** was unique to the sterile soil, suggesting that it is an abiotic hydrolysis product. Triazine ring-opened degradation products analogous to **8** have been reported for other SU herbicides (prosulfuron,[24] thifensulfuron-methyl[26] and chlorsulfuron[27]) in hydrolysis studies. A triazine ring-opened degradation product of metsulfuron-methyl was reported in an aqueous hydrolysis study at pH 5 and in the sterile soil in a soil degradation study.[9,12] Both hydrolysis and soil studies reported the same triazine ring-opened degradation product with an elemental composition of $C_{13}H_{14}N_4O_8S$ (that is equivalent to metabolite **8** in our study), and two structures were proposed.[28] Neither of these proposed structures has proven to be correct in light of our unequivocal synthesis of the reference standard **8** and its comparison with the observed metabolite in the sterile soil by LC/MS and HPLC.

Metabolite **8** was not observed in the non-sterile soil. There are two possible explanations. First, metabolite **3** may be further degraded preferentially into metabolite **4** by microbial transformation instead of metabolite **8** by chemical hydrolysis (Fig 1). The microbial enzymatic reaction from metabolite **3** to **4**

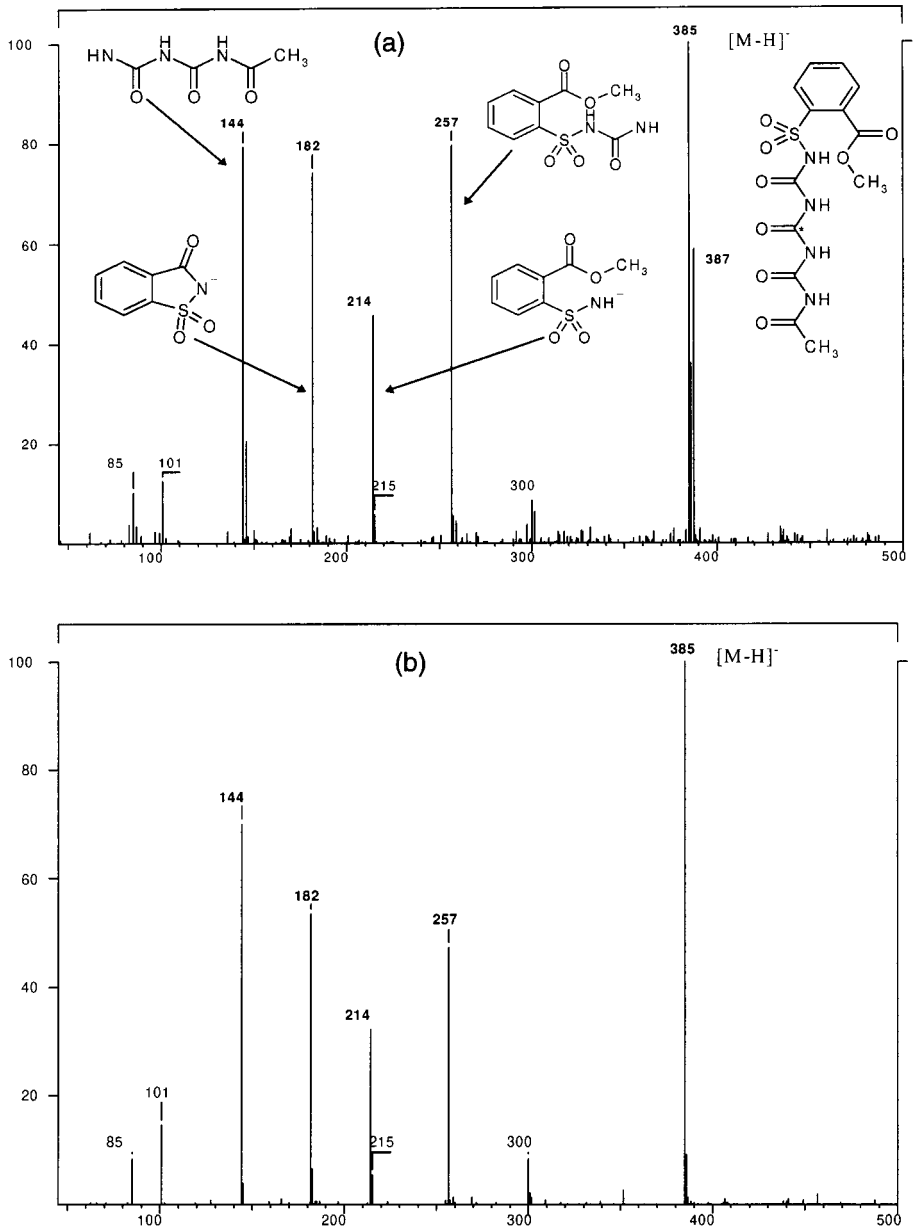

Figure 8. Mass spectrum of metabolite **8** and the reference standard (* denotes site of ^{14}C). (a) Negative ion CID (−30 volts) mass spectrum of metabolite **8** (triazine label). (b) Negative ion CID (−30 volts) mass spectrum of the reference standard.

may be faster than the hydrolysis reaction to **8**. Second, metabolite **8** may have formed but degraded microbially to undetectable levels by the time the samples were taken.

Metabolite **4** appears to be a biotransformation product since it was not detected in the sterile soil. We believe that this is the first report of such a metabolite for metsulfuron-methyl that has been identified and confirmed by comparison with a synthesized authentic reference standard. An analogous metabolite has been observed in a chlorsulfuron soil dissipation study.[29] A mechanism for the formation of **4** can be proposed as follows: The first step appears to be *O*-demethylation of the triazine ring to form metabolite **3**, since this metabolite was observed initially, then declined while the presence of **4** gradually increased (Fig 6). Enzyme-mediated hydrolytic bond cleavage of two of the triazine ring C–N bonds is necessary to arrive at the carbamoyl guanidine structure of **4.**

4 CONCLUSIONS

Metsulfuron-methyl degraded rapidly in an acidic soil in the dark at 20°C (±2)°C under aerobic conditions. The estimated DT_{50} and DT_{90} values of metsulfuron-methyl in the non-sterile soil using a biphasic model were approximately 10 days (9–11 days, 95% confidence limits) and 44 days (40–50 days, 95% confidence limits), respectively. This result is consistent with those previous reported.[14,30]

The principal degradation product after 12 months is carbon dioxide, which accounted for approximately 48% AR and 23% AR in the [*phenyl*-U-^{14}C]-

and [*triazine*-2-^{14}C]-metsulfuron-methyl treatments, respectively. The major routes of degradation are *O*-demethylation, sulfonylurea bridge cleavage and triazine ring opening. Two triazine ring-opened products, metabolite **8** in the sterile soil, and metabolite **4** in the non-sterile soil, were found in this study. The acetyl triuret **8** and analogous compounds have been observed in the hydrolysis of metsulfuron-methyl and related SU herbicides, as discussed previously, while the carbamoyl guanidine **4** has not been previously reported for any metsulfuron-methyl study. Microbial metabolism best explains the formation of **4** while chemical hydrolysis leads to the formation of **8**. The potential for soil persistence of sulfonylurea herbicides containing the triazine moiety breakdown products has been previously the subject of speculation;[31] however, using sensitive LC/MS technology, the structures of major triazine ring breakdown products have been definitively elucidated. The results indicate that triazine ring opening did occur and that hydrolytic and microbial mechanisms were operable.

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